NOCARDIA ISOLATED FROM SOIL AND ITS POTENTIAL TO INDUCE MASTITIS IN GOATS

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DEDICATION

I dedicate this work the soul of my mother, kind

Father, lovely sisters and brothers.

PREFACE

This study has been completed at the department of Preventive Medicine and Public Health and the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum .

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ABSTRACT

This Study was conducted to isolate and identify nocardiae from soil samples collected from different sites of Sudan and to use these isolates to experimentally induce mastitis in Nubian goats.

Eight soil samples were collected from the following sites in Sudan, Hag A bdalla, Rehaid Elbridy, Elgadareif, Eldalanj, Kassala, Shambat. The samples were cultured on tryptic soy agar (T. S. A) and incubated aerobically at 37°C for up to 5 days.

Nine *nocardia* isolates were recovered from the cultured samples. The isolated strains were phenotypically characterized using morphological, cultural, biochemical and mycolic acid analysis.

The phenotypic characterization of the isolates classified them into 3clusters: Cluster 1 was typically *N. brasilliensis*, Cluster 2, was characteristically *N. asteroides* and Cluster 3 was identified as *N. farcinica*. Two of the isolates were not identified as typical nocardia although they were morphologically resemble nocardia.

The test for viability *of N. farcinica* in contaminated fence soil established that, under natural conditions, N*. farcinica* could remain viable in soil for 30 months.

From the isolated Nocardiae *N. farcinica* AB14, *N. farcinica* AB15, and a control strain *N. farcinica* SD1800 were used for

experimental induction of mastitis in two Nubian goats. The two strains caused mild mastitis associated with high leukocytes count with no systemic reactions. The organisms were constantly execreted in the milk and were demonstrated in smears and cultures.

The two infected goats were treated with broad spectrum antibiotics Neomastipra intramammary infusion but did the infection did not respond to it. After the end of the withdrawal period of Neomastipra, treatment of mastitis was tried using Gentamast but also failed to get rid of infection. After the withdrawal period of both drugs, nocardiae were recovered in pure cultures on T.S.A.

The study concluded that Nocardiae are readily available in soil samples and could cause mastitis in goats. Further, the finding showed that Tryptic Soy Agar medium with combination of 5µg /ml tetracycline, 50µg /ml nystatine and 5%NaCl was the best medium for isolation of nocardiae from soil.

ملخص ألاطروحه

هذه الدراسه صممت لعزل وتعريف البكتيريا من جنس النوكارديا في عينات من التربة جمعت من أنحاء مختلفه من السودان ودراسة مقدرة هذه العزلات لاحداث التهاب الضرع تجريبيا في الماعز النوبيه.

تم جمع ثماني عينات من التربة من مناطق مختلفة في السودان شملت حاج عبد الله، رهيد البردي، القضارف، الدلنج، كسلا وشمبات. تم زرع هذه العينات في وسط أجار التربتك صويا و تحضينها في في درجة حرارة 37°م.

تم عزل تسعه عترات من النوكارديا من العينات المذروعه.

درست الخواص المظهريه للمستعمرات البكتيرية للعترات المعزوله (9) وعرفت خواصها الكيموحيوية وقد صنفت تبعاً لذلك الي 3 مجموعات شملت مجموعة النوكارديا برازيلينسس، مجموعة النوكارديا أستروبسس ومجموعة النوكارديا فارسينكا. أثنين من العزلات لم تصنف كنوكارديا بالرغم من أن خواصها المظهرية تشابه النوكارديا.

لمعرفة مدي مقدرة النوكارديا المعزولة من عينات التربة الملوثة ببكتريا النوكارديا فارسينكا علي إحداث إلتهاب الضرع في الماعز، حقنت اثنتين من الماعز النوبيه ببكتيريا النوكارديا فارسينكا و تمت دراسة الأمراضية فيهما.

نتائج الأصابة المخبرية أظهرت أن عترات النوكارديا فارسينكا لها القدرة علي أحداث التهاب الضرع وتم عزل البكتريا بصورة نقية وكان افرازها متقطعاً ولم تحدث أي تغييرات جهازية.

أخضعت الماعزتين للعلاج بالمضاد الحيوي واسع الاطيف نيوماستبرا موضعياً ولكن المضاد الحيوي لم يكن فعالاً في التخاص من الأصابة حيث كانت نتائج الفحوصات البكتيرولوجية موجبة للنوكارديا طوال فترة العلاج بالنيوماستبرا، لذلك عقب إنقضاء فترة with drawl period أختبر الجينتاماست لعلاج الألتهاب و لكنه أيضاً لم يفلح في التخاص من الأصابة حيث عزلت النوكارديا من العينات التي جمعت خلال فترة العلاج بالجينتاماست،هذه النتائج أظهرت أن إلتهاب الضرع بالنوكارديا صعب العلاج ويحتاج للمزيد من البحث.

من هنا نستخاص أن وسط أجار التربتك صويا مع خليط من 5ميكروجرام من التترسيكلين، 50 ميكروجرام من النيستاتين و5%من كلوريد الصوديوم من البيئات الممتازة لعزل النوكارديا من التربة.

كذلك أظهرت الدراسة أن عزلات النوكارديا من التربة لها المقدرة على احداث التهاب الضرع فى الاغنام.

INTRODUCTION

Resent data from the Animal Wealth Sector Performance Report, Federal Ministry of Animal Resources in the year 2000 reported 37 million heads of goats are in the Sudan.

Many infectious diseases affect goats in Sudan. They include goat pox, contagious caprine pleuropneumonia, heart water, rinderpest, internal and external parasites and mastitis (Anon, 1981).

Mastitis is considered as one of the major diseases of goats in Sudan (Abu Samra *et al.,* 1988). It causes serious economic losses to goat industry but no data is available to describe the extent of loss.

Mastitis in goats is caused by many infectious agents including *Staph. aureus*, *Str. agalaciae, Str. dysgalactiae, Str. pyogenes, Mycoplasma agalatiae , Mycoplasma mycoycoides var. mycoide, Yersinia pseudotuberculosis,* and nocardiae (Blood *et a*l., 1983).

Member of the genus *Nocardia* especially *N. asteroidses* and *N. farcinica* were reported as infrequent causal agents of mastitis in dairy cattle and goats (Rodostitis *et al.,* 2000). In Sudan there were numerous reports of mastitis in goats caused by *Nocardia* spp. (Daffa and Gharib, 1958, Ibrahim, 1968; Maldonado *et al.,* 2004).Bovine

mastitis due to nocardiae have also been described in the Sudan (Shigidi and Mamoun, 1981; Hamid *et al.,* 1998).

Two studies described the isolation of nocardiae from soil in Sudan Ajello *et al*., (1979) and Sid Ahmed (2001). But studies from other part of the world described the isolation of nocardiae from soil (Orchard and Goodfellow, 1980). Tryptic Soy Agar (T.S.A) supplemented with 5µg/ml tetracycline and 50µg/ml nystatine was found to be the best medium for selective isolation of nocardiae from soil (Sid Ahmed, 2001).

There were some studies to isolate *Nocardia* spp.from clinical samples and from soil but to our knowledge this is the first study in the Sudan that investigated the pathogenicity of soil isolates of *Nocardia* spp. to Nubian goats.

Objective:

The main objectives of the present study was

1- To isolate *Nocardia* and *Nocardia*- like organisms from soil

2- Identify these isolates by conventional methods and mycolic acid analysis

3- Study the viability of *N. farcinica* in goat's bedding

4- Experimentally induce mastitis in goats using the soil isolates of *N. farcinica*.

CHAPTER ONE

LITERATURE REVIEW

1.1 Goats of Sudan

The goat population of the Sudan was estimated approximately about 11millions (Yassin and Hussein, 1985). Recent data from the Animal Wealth Sector Performance Report, Federal Ministry of Animal Resources in 2000 reported 37 million heads of goats in the Sudan.

1.1.1 Breeds

At least six methods of classifying domestic goat based on origin, utility, body size, ear shape and ear length exist (Payne, 1990). Devandra and Burns (1983) classified goat into three categories on the basis of body size.

There are many goat breeds in the Sudan but the main breeds are the Nubian, Sudanese desert and the Nilotic (Devendra,1983).

1.1.2 Sudanese Nubian goats

The Nubian breed, also referred to as local Nubian in Egypt, has curved horns in both sexes and small ears which may rector dropping. The hair is long, and the color black or black and white (Devendra, *et al.,* 1977). It is one of the African breeds that has been selected for milk production. Their average daily milk is 1-2kg (Devendra *et al.,* 1983). They are mainly kept in urban areas and often stall fed.

It is certainly not the Nubian supposed to contribute to the ancestry of the Anglo-Nubian (Devendra*, et al*., 1977). It is unfortunate that the name Nubian has been applied to both type of milking goats developed in this area. Ancient Nubian occupied an area now in southern Egypt (Wawat) and Northern Sudan (Kush).

1.1.3 Sudanese desert goats

These goats belong to the arid area of northern Sudan. Both sexes are horned, the horns of the male being longer, twisted and projecting laterally, the pendulous ears are of moderate size. The desert goats have long legs and short fine gray coats which are adaptation characteristics to arid regions (Devendra, *et al*., 1977).

They are primary producers of meat and hides These goats have been shown to produce more meat than Sudanese desert sheep. The breed is very prolific, producing a high proportion of twins, with production of 9 - 10 kids (Devendra, *et al.*, 1977).

1.1.4 The Nilotic goats

A typical example is the type found in southern Sudan. The male has short horns, and females have either very short horns or none at all. These goats show some similarities with the small east African goat (Devendra*, et al*., 1977).

1.1.5 System of management

In Sudan, five systems of management appropriate to goats are available:

- Village system.
- Extension system.
- Semi – intensive system.
- Very intensive system.
- Integrated with cropping system.

Extension systems are not particularly common by comparison. The village extension and integration with the cropping systems are traditional.

1.1.6 Importance of goats

The goats are particularly important animals in subsistence agriculture because of its unique ability to adapt and maintain itself in harsh environment. It is believed that the goat was probably the second animal to be domesticated after the dog (Devendra, *et al*., 1977).

Goats are multi purpose animals; producing milk, hides and hair. In addition to their main function, goats are important for a number of miscellaneous reasons. They considered an investment against the failure of cash crops among many farmers, and in many communities they have especial place in local custom, religion and festive occasions

(Devendra, 1966). They are also used for the production of manure and more recently convenient experimental animal for metabolic studies.

In the past there have been some disagreements as to the value of goats because of a widely held belief that the damage they do to the trees and vegetation especially in arid regions outweight their use as producer of meat, milk and with controlled management they can be a great help for agriculture development and food production.

1.2 Mastitis

The term mastitis refers to the inflammation of the mammary gland regardless of the causes. It is characterized by physical, chemical and usually bacteriological changes in the glandular tissue (Blood, *et al.,* 1983).

The most important change in the milk is discoloration, presence of clots, and elevated leukocytes count. Although there is swelling, heat, pain and induration in the mammary gland in many cases, a large proportion of mastatic gland are not readily detected by manual palpation or by visual examination of milk using a strip cup (Blood, *et al.,* 1983).

In the Sudan, mastitis is one of the main diseases of goats (Abu Samra, *et al.,* 1988). It was first reported in the Sudan in 1953 (Annual Report of the Sudan Veterinary Service). Since then, it had been

described as being fairly common (Annual Report of the Sudan Veterinary Service, 1953-1955 and Animal production, 1956-1957).

The prevalence of mastitis in dairy herds in the Sudan was first thoroughly investigated by Wakeem and El Tayeb (1962). The investigation was carried out to determine the incidence, prevalence rate of infections, the causative agent and response to control efforts which included treatment.

1.2.1 Mastitis in goats

Mastitis is common disease in lactating goats and is caused by a number of different types of pathogenic bacteria (Devendra, *et al.,* 1983). The organisms most frequently involved in goats mastitis in Sudan are *Staphylococcus aureus*, *streptococci, Corynebacterium pyogenes, mycoplasma*, *nocardia* and coliforms (Ibrahim 1968; Yassin, 1985).

With the exception of mycoplasma species which can invade the mammary gland from the blood stream most organisms which cause mastitis enter the gland through the teat canal (Quinn *et al.,* 2002).

Staphylococcal mastitis in goats is characterized by gangrenous and rapid necrosis of mammary tissue (Gamal Eldin, 2003). Atrophy of the udder is often associated with mycoplasma mastitis (Prasad, *et al*., 1985).

Mixed infection was more common among goats showing clinical evidence of infection than among those with subclinical infection (Ibrahim, 1968).

It is important to remember that milk from goats can appear relatively normal even with severe inflammatory changes in the udder (Quinn, *et al.,* 1999).

1.2.2 *Nocardia* mastitis

Nocardial mastitis is important problem in cattle and goats (Dwisht, *et al., 1999*). Nocardial mastitis is characterized by anorexia, fever and extensive granulomatous lesions in udder and lungs (Dafaala and Gharib, 1958). *N. asteroides* has been found in mastitis in goats (Dorsey and James, 1973). *N. asteroides* has been reported as a cause of goat mas*titis* in Sudan by Dafaala and Gharib (1958) and Maldonado, *et al*. (2004). Nocardia was isolated from the udder secretion of 10 years old goat.The disease was chronic affecting one half of the udder, which became in the form of a big tumor-like mass with multiple chronic abscesses (Ibrahim, 1962). *N. asteroides* mastitis is characterized by anorexia and granulomatous lesions in udder and lungs (Dafaala and Gharib 1958, Megid *et al*., 1990; Gamal Eldin, 2003). The importance of recognizing mastitis due to *nocardia* lies in the fact that *nocardia*

especially *N. asteroides* and *N. farcinica* are resistance *in vitro* to the most common antibiotics (Hamid *et al.,* 1998; Patrick, *et al*. 2003).

Twelve out of the 84 (14.3%) samples of bovine mastitic milk collected from Khartoum, Gezira and Western Sudan revealed the presence of nocardia and nocardia- like actinomycetes.The distribution of the 12 positive cases was 10 cases from Khartoum state, 1 case from El Gezira state and 1 case from Western Sudan (Ahmed, 2003*). N. farcinica* was isolated from zebu cattle suffering from mastitis in western Sudan (Hamid, *et al.,* 1998) *N. farcinica, N. asteroides* and *N. brasilliensis* were isolated from goat in Khartoum State (Gamal El Din, 2003; Maldonado, *et al*., 2004).

1.3 Epidemiology

Pathogenic nocardiae occur worldwide suggesting constant exposure. In humans, the disease is associated largely with immuno deficiencies (Dwight and Yuan 1999). The use of dry cow products containing neomycin, including 2 specific dry cow products, was strongly associated with Nocardia mastitis in herd. Other factors which increase the risk are the high production, larger herd size, and large percentage of cows treated with dry cow products (Ferns, *et al.,* 1991).

Pathogenic nocardiae had been isolated from soil samples around Khartoum State (Sid Ahmed, 2001). Pathogenic nocardiae had been also isolated from milk samples of cows from Khartoum, El-Gezira and White Nile (Ahmed, 2003) and likewise isolated from milk samples from Goats (Gamal Eldin, 2003).

1.4 Diagnosis of mastitis

Detection of mastitis is generally based upon some manifestation of the inflammation (changes in the udder or milk) (Morin and Hurley, 1993).

The common screening tests used in the diagnosis of bovine mastitis include clinical examination, cow side test, rapid mastitis test, somatic cell count and bacteriological examination. These tests have been studied by various workers to see if they were applied to caprine mastitis. Nesbakken (1976) reported that cell count of milk goat is diagnostic.

1.4.1 Cow side test

Many simple tests performed on the farm, or more complicated laboratory tests are used to diagnos mastitis in individual cows or herd basis (Anon, 1987). Clinical mastitis may be detected by examination of the udder for warmth, swollen quarter, which are indicative of acute mastitis, or for hard, atrophied and fibrotic quarters, indicating

permanent damage caused by chronic mastitis. The strip cup test in these cases is not sufficiently sensitive to detect subclinical mastitis.

1.4.1.1 California Mastitis Test (CMT)

California mastitis Test (CMT) is another side test that can be used for the detection of mastitis. This is an indirect test that grossly measures the amount of DNA, primarily function of the nucleated white blood cells in the milk (Quinn, *et al*., 1994). The basis of the CMT is the reaction which occurs when the reagent comes into contact with the increased quantity of cell material derived from increased number of somatic cell in the milk (Bramly, 1975).

According to Siddique *et al.* (1988) there was a correlation of CMT with the number of neutrophils/ml and that CMT could be applied to test goat milk. The CMT was commonly used for detection of mastitis and has proved to be highly efficient (Blood et *al.*, 1983). It is more sensitive than the strip cup test and enables subclinical mastitis to be detected.

1.4.1.2 PH indicator papers

These test strips detect the increased alkaline pH in quarters with mastitis. Normal milk has a pH of a proximately 6.5 to 6.7, where as the pH of mastitic milk approaches plasma pH of 7.4 (William, 1995).

1.4.1.3 Palpation of the udder

This is very helpful in detecting areas of fibrosis associated with chronic sub clinical *Staphylococcus aureus* infection. Palpation also is valuable following resolution of acute clinical mastitis to detect glandular changes that might be associated with infraction, abscessation or chronic infection (Rodostits, *et al.,* 1996).

1.4.1.4 Somatic cell count (SCC)

This has become the most widely used index of the infection in individual cows and herds (Bartlett *et al.,* 1992). Infection is the major factor that elevates the somatic cells count (Ersikine, *et al.,* 1980). Somatic cells consist primarily of leukocytes that are present in the udder in response to infection. Somatic cells also include epithelial cells which make the internal lining of the mammary gland tissue and are normally replaced during the event of lactation (Harmon and Langlois, 1986). When the udder or teat is severely injured there is large increase in SCC. Neutrophils increase dramatically to compose the majority of cells and may increase to 95% of the somatic cell count with infection of the gland (Kirk, 1984). The direct microscopic somatic cell count (DMSCC) is the procedure of evenly spreading a measured volume of milk over a calibrated area of a microscope slide, staining the film and counting somatic cells with in specific area of the film. The count is then

converted to cells per milliliter (ml) by a factor which is determined by magnification and areas counted (Packard *et al.,* 1992).

1.4.2 Laboratory methods

1.4.2.1 Culturing

Bacteriological culture of milk samples from individual quarters is required to determine the aetiological agents involved (Anon, 1987). In herds with large number of sub clinical cases of mastitis a reliable diagnosis can be made by culturing samples of milk from selected cows on basis of increased CMT scores or SCC. Most of the bacterial pathogens causing mastitis grow on ox or sheep blood agar. A Sabarourd dextrose agar plate can be inoculated if a fungal pathogen is suspected (Quinn, et al., 1994; Carter, 1996).

1.5. Isolation of nocardiae from soil

The classical method of isolating nocardiae from soil is the paraffin baiting technique (Gordon and Hagan, 1936). This method employs glass rods coated with paraffin wax dipped into a carbon free medium inoculated with soil suspension. The method demonstrated the presence of *N. asteroides, N. brasiliensis* and *N. cariae* in soil samples from United States of America and India (McClung, 1960; Kurup and Sandhu, 1965; Kurup *et al.,* 1968; Kumar and Mohapatra, 1968). The method was also used to isolate *N. asteroides* from clinical materials

(Mishra and Rand hawa, 1969; Kurup *et al.,* 1970). Nocardiae have also been isolated using a technique that involves the inoculation of guinea pig and hamster testicles with soil suspension with added penicillin and streptomycin (Conti –Diaz, *et al.,* 1971). Neither the animal inoculation nor the paraffin baiting technique was of value in quantitative studies and the latter was of limited use for the isolation of the organisms because of the difficulties of separating nocardiae from contaminating bacteria and fungi (Valerie *et al.,* 1974).

Laboratory strains of *Nocardiae* were tested for their *in vitro* susceptibility to 52 antimicrobial agents in an attempt to test those compounds for their usefulness in selective isolation of nocardiae from natural habitats. The method was found to support the growth of nocardiae with few bacterial contaminants (Orchard and Goodfellow, 1974).) Used Diagnostic Sensitivity Test Agar (DST) supplemented with actidione and nystatin each at a concentration of 50μg/ml. Sterile aqueous solutions of chlortetracycline hydrochloride, demethyl chlortetracycline hydrochloride and methancylcline hydrochloride were added to the basal medium to give final concentration of 45,5 and 10μg/ml, respectively. The antibacterial antibiotics were used singly or in combination to determine the highest number of Nocardia-like bacteria that could be obtained. Soil samples were collected and stored

at 4°C before testing for the presence of nocardia. Preparation of soil suspensions for each sample initial dilution was prepared by adding 1g of soil to 10ml of sterile 25% (v/v) strength Ringers solution, the 10^{-1} dilution were shaken for 30 minutes on Griffin shaker (Griffin and George Ltd., Manchester). After agitation, 1mL of each Soil suspension was pipetted into 9ml of diluent to give a 10^{-2} dilution. Surface spread plates were made by spreading over dried DST plates containing the various antibacterial antibiotics, and over control plates containing the anti-fungal agent only. The plates were incubated at 37°C and examined after 7-14 and 21 days (Orchard and Goodfellow, 1974).

Different methods and media have been used for the isolation of *Nocardia* spp. in bulk tank milk samples and from samples of milk from individual cows. *N. farcinica* strains isolated on Lowenstein Jensen slants which were incubated at 37°C under aerobic condition (Hamid *et al.,* 1998). Trypticase soy agar supplemented with 5% (v/v). defibrillated bovine blood and gentamycin sulphate (25mg/ml) was reported as a good selective medium for the isolating and identifying *Nocardia spp.* present in milk samples (Lynch, 1990).Blood agar containing 25mg gentamicin per liter (Schoon derwoerd *et al.,* 1990) were used to isolate *nocardia* and reduce back ground bacteria.

N. asteroides was isolated from milk on sheep blood agar at 37°C under aerobic condition (Wendlt, *et al.,* 1969).

Clinical material such as bronchial washing, sinuses discharge and biopsy, need to be examined as soon as possible to prevent over growth by contaminants. Fluid material can be examined in wet mount under the microscope with out staining. Gram-positive branching filament can be seen at high magnification, the filament may show evidence of fragmentation into rods and coccoid- like element (Goodfelow, 1998). Acid- fast is usually more pronounced in clinical than cultural material.

Several general-purpose media can be used to isolate *Nocardia* from clinical material they include brain–heart infusion, Sabouraud dextrose, Yeast extract-molt extract agar and diagnostic sensitivity test agar (Goodfellow, 1998).

1.6 Clinical and pathological findings

Nocardiae include many pathogenic species, *N. asteroides* cause*s* chronic granulomatous mastitis in cattle. In sheep, goats, pig and other animal it causes less frequent infections, such as pneumonia, mastitis and lymphadenitis (Quinn, 1994).

In goats, mastitis is characterized by anorexia, fever, and granulomatous Lesions in udder and lungs (Megid, *et al.,* 1991). *N.*

farcinica caused acute mastitis in goats and udder secretions changed to yellowish viscous clotted secretion with high leucocytes counts (Gamal El din, 2003). In goats the affected half of the udder become big and tumor like mass with multiple chronic abscesses-the mammary secretion had almost completely stopped and change to whey-tike secretion contained large caseous clots (Ibrahim, 1962). In cattle, the more severe form of the disease was characterized by high body temperature and a rapidly progressive fibrosis of the udder. Spread of the infection to lungs and supra mammary lymph nodes were observed. The less sever form usually led to progressive fibrosis of the affected quarter (Pier, *et al.,* 1961). In cattle *N. farcinica* causes acute mastitis with fever, anorexia diarrhea, lacrimation, and milk secretion was yellowish in color with clots and flakes, anorexia and high leukocyte count (Ahmed, 2003).

In cattle the mammary secretion of affected glands was grey with yellowish-grey clumps, abscess content was viscous, yellowish grey and mixed yellowish–green solid material and white mycelia clumps (Hillermark, 1960). The fibrosis may be diffuse but usually in the form of discrete masses 2-5cm in diameter. Badly affected gland may be rupture (Rodostitis *et al*., 1983).

1.6.1 Antimicrobial susceptibility of Nocardia isolated from

mastitis

Strains of *N. farcinica* isolated from milk in Khartoum State were found sensitive to chloramphenicol, neomycin and gentamicin but were resistant to penicillin, ampicillin and sulpha-methazole trimethoprium (Gamal Eldin, 2003).

Strains of *N. asteroids* varied in their sensitivity, but in general they were relatively sensitive to doxycycline, minocycline, erythromycin and streptomycin (Lerner and Baum, 1974). Nassal (1967) reported that *N. asteroides* was sensitive only to erythromycin and kanamycin, while resistant to penicillin and oxytetracycline. *N. asteroides* was found sensitive to gentamicin, neomycin and streptomycin, and resistant to ampiclline, chloramphenicol and oxytetracycline (Savalia and Khir, 1990).

1.6.2 Treatment and control

Nocardiae especially *N. asteriodies and N. farcinica* are resistance, in vitro, to the most common antibiotics (Hamid and Goodfellow, 1997). Isoniazid was found to be effective in the treatment of acute mastitis and in prevention of the development of acute mastitis in infected cows in late pregnancy, but didn't eradicate organisms from the udder (Wendt, *et al.,* 1969). *N. farcinica* was reported resistant to intramammary infusion containing neomycin, penicillin and streptomycin

(Neomastipra) and the treatment didn't reduce or eradicate the organism from the udder (Gamal Eldin, 2003). A case of mastitis caused by *N. asteroids* was reported in a cow and the in vitro drug sensitivity testing indicated that *N. asteroids* isolated from that cow was sensitive to gentamycin neomycin and streptomycin and resistant to ampicillin, chloramphenicol and tetracycline (Savalia, 1990).

Outbreaks of nocardia mastitis in cows were controlled by culling all the infected cows from the herd (Wendt, 1969). Better hygiene management and treatment practices would reduce the incidence of mastitis (Triphi et al., 1993).

Ahmed (2003) reported that although treatment with gentamycin improved the condition and the systemic reactions disappearedbut milk secretion was still positive for Nocardiae.

1.7 The genus Nocardia

1.7.1 Definition

Nocardia are aerobic catalase–positive non-motile actinomycetes. They are Gram- positive and are typically acid- alcohol fast at some stage of growth cycle.

The nocardiae have extensively branched vegetative hyphae, 0.5- 1.2µm in diameter, that grow on the surface of and penetrate agar media; these hyphae often fragment in situ or on medical distribution

into rod-shaped to cocco-bacilli forms (Goodfellow 1998). The wall envelope of nocardia contains mycolic acid with 44- 64 carbon atoms. The polysaccharide fraction of the wall is rich in asteroids and glactose (Goodfellow, 1998).

1.7.2 Habitat

Nocardiae are a widely distributed and are abundant in soil (McNeil et al., 1994). Which are predominantly saprophytic (Goodfellow, 1998). But they also include species forming parasitic association with animals and plants. They occur in a wide range of man-made natural habitats including activated sewage, sludge soil, water and tissue of plants and animals including humans (Goodfellow, 1998).

1.7.3 Morphology and cultural appearance

The only constant morphological feature of Nocardiae is their ability to form filamentous, branched cells which fragment into pleomorphic rod- shaped and coccoid elements. Most Nocardiae produce caratenoid-like pigments that result in colonies with various shades of orange, pink, red or yellow. Soluble brown or yellowish pigment may be produce. Colonies of Nocardiae may be smooth or granular and irregular wrinkled or heaped (Goodfellow, 1998).

1.7.4 Cultural characteristics

Nocardiae have an oxidative type of carbohydrate metabolism. They can use a diverse range of fatty acid, hydro carbons, steroids and sugar as source of carbon for energy and growth (Goodfellow, 1998). Most strains grow on media containing simple nitrogen source such as amino acids, ammonium and nitrate and on media supplemented with casein, meat extract, soya or yeast peptones and hydrolysates. They grow well between 25-37°C, some strains reach stationary phase in 3-7 days; others grow more slowly (Goodfellow, 1998).

1.7.6 Resistance to physical and chemical agents:

Nocardiae are quite resistant to heat and desiccation. Most strains can with stand 50° C for 8 hrs (Goodfellow, 1998). Antimicrobial susceptibility testing is difficult as results may be influenced by factors such as pH, inoculum's size, composition of agar assay media and by spontaneous drug degradation due to the slow growth rate of the organism (Goodfellow 1998).

Strains of *N. farcinica* are resistant to cephamadol, cefotaxime and tobramycin. Antibiotic sensitivity of *N. asteroides* varies with the stage of growth (Goodfellow, 1998).

1.7.7 Identification and differentiation of Nocardiae

Nocardiae are most easily distinguished from Actinomadura, Streptomyces and other Sporactinomycetes as they alone have whole

organism hydrolysates containing mycolic acid (Goodfellow, 1998).

Qualitative evaluation of mycolic acid can be easily and quickly achieved by thin layer chromatography. A combination of chemical, morphological and physiological tests are necessary to distinguish between mycolata genera.

The phenotypic identification tests recommended by number of investigators are inadequate and only give presumptive identification (Goodfellow, 1998).

The use of polymerase chain reactin (PCR) coupled with restriction endonuclease analysis of PCR products has been focus as recent interest for the separation of Nocardia spp.. (Goodfellow, 1998). This approach promises to provide a rapid, sensitive and effective way of identifying clinically significant Nocardiae. Kiska, *et al*. (2002) reported that a single method could not identify all Nocardia isolates to the species level; therefore, a combination of methods was necessary.

1.7.8 Clinical Significance of Nocardiae

Nocardiae include many pathogenic species that affect man and animals. Infection may occur by inhalation, contaminated wounds and traumatic implantation.

N. asteroides causes granulomatous mastitis in cattle (Pier, *et al .;* 1961). *N. farcinica* causes mastitis in cattle (Hamid, *et al.,* 1998).

In goats *N. asteroides* causes granulomatous mastitis (Megid, 1990). Cutaneous actinomycosis and nocardiosis were seen in dogs (Kirpenstin and FingLand 1992).

N. africana strains were isolated from patient with pulmonary infection (Hamid *et al.;* 2001*). N. brasiliensis* was isolated from various kinds of infection, including mycetoma (Goodfellow and Lechevalier, 1989). *N. transvalensis* was isolated from mycetoma of the foot. *N. asteroides* (16 strains) were isolated from fish (Chen and Wang, 1993). Various nocardial infections were reported in human after surgical operation (Vandime, *et al.,* 2001).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Soil samples

Soil samples were collected from different sites of Sudan. The sites were chosen to give a good representation to the Sudan with it is geographic niches.

The names and states of these sites are given in Table (1).

2.1. 1 Sample collection

Soil samples (10 g) were collected from freshly exposed surface of each site. The samples were placed in sterile universal bottles, then transported to the laboratory for culture.

2. 2 Culture Methods

Tryptic soy agar medium was used for isolation of nocardiae (Difco Laboratory, Deteroit Michigan, USA). The medium was supplemented with a combination of NaCl 5%, 5µg/ml tetracycline (tetracycline HC 250µl Bipamipharma Laboratory Ltd., Sudan) and 50µg/ml nystatine (Merck, 64271 Darmstadt, Germany).

Table 1: Sites of soil samples

State	Site	No. Samples
Gezira	Hag A bdalla	1
Southern Darfour	Rehaid Elbridy	1
Gedearif	Elgadareif	1
Southern Kordofan	Eldalanj	1
Kassala	Kassala	1
Khartoum	Shambat	4

Based on different trails performed during this study, T.S.A with this combination (NaCl, tetracycline, nystatine) was taken as a suitable selective medium for the isolation of *Nocardia spp*. from the soil samples. T.S.A medium was also used for subculture and preservation of the isolates.

2. 2. 1 Preparation of soil samples

For each sample, the initial dilution was prepared by adding 0.1g of soil to 10ml of sterile normal saline. The 10^{-1} dilution was shaken vigorously for 30 minutes, allowed to precipitate and the supernatant was used for culture.

2. 2. 2 Primary culture

Cultures were made by spreading 0.1 ml soil suspension aseptically on T.S.A plates containing 5µg /ml tetracycline, 50µg /ml nystatine and NaCl 5%. The plates were incubated aerobically at 37°C and examined daily for up to 7 days for growth of nocardiae.

2.2.3 Purification of culture

Isolated nocardia – like colonies from the primary culture were picked with a wire loop and streaked on the surface of a fresh plate of T.S.A medium. The growth was identified macroscopically by the presence of identical discrete colonies along the streak lines.

Nocardia colonies were identified by the characteristics described by Goodfellow (1998).

2.2.4 Preservation of culture

2.2.4.1 Frozen glycerol suspension

Colonies of purified culture were kept in sterile 20% glycerol as described by Williams *et al;* (1983). Heavy cell biomass from young culture was transferred into sterile tubes containing 1 to 5 ml of 20% glycerol.

The cell suspension was kept in deep freezing at –20°C.

2.2.4.2 Subculture

Subculture was made by streaking a loopful of purified isolates on T.S.A plates. The plates were incubated aerobically at 37°c for 48 hors. The plates were cellutaped and kept at room temperature.

2.2.4.3 Slants

Growth on T.S.A slant (Bijoux, Mc carteny or Universal bottle) remained viable for several months and was subcultured every three months to ensure viability of nocardiae. Cultures were kept at room temperature.

2.3 Identification of soil isolates

Identification of isolates was done according to Quinn et al. (1999) based on clonial characteristics microscopic examination and biochemical reactions.

2.3.1 Colony morphology and staining reaction

Colony characteristic were studied using Gulucose yeast extract agar and Tryptic soy agar plates after incubation at 37ºC

Smears made from pure colonies and stained with modified Ziel Neelsen and gram stains (Appendix B) and then examined microscopically for the presences of branching filaments or coccobacilli forms.

2.3.2 Biochemical tests

All biochemical tests were done according to Barrow and Feltham (1993).

2.3.2.1 Urease tests

Urease medium slants (Appendix A) were inoculated with each of the test organisms, incubated aerobically at 37°C and examined daily for seven days. Development of a pink color indicated a positive result.

2.3.2.2 Catalase Test

A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean microscopic glass slide. A small amount of the test organism was mixed with H_2O_2.

Production of gas bubbles indicated the release of O_2 by catalase enzyme from the bacteria, which was taken as a positive result.

2.3.2.3 Casein degradation

A typical and well isolated nocardiae-like colony were picked with a wire loop and streaked on the surface of a fresh plate of casein agar (Appendix A). The plates were incubated at 37°C and examined daily. The positive result gave a clear zone around the growth indicating the utilization of casein by grown organisms.

2.3.2.4 Tyrosine degradation

A loopful of test organism was cultured on a dry plate of tyrosine agar (Appendix A). The plates were incubated at 37° C and examined daily for development of a clear zone around the colonies indicative of tyrosine degradation.

2.3.2.5 Starch degradation

The test organisms were cultured on starch media (Appendix A). Plates were incubated at 37° C and examined daily. The result of the utilization of starch is read after the addition of lugol's iodine.

Organisms utilizing starch showed a zone of clearance round the growth.

2.3.2.6 Xanthine

The test organisms were cultured onto fresh plates of xanthine agar (Appendix A). The positive result gave a clear zone around the growth, indicating the utilization of xanthine by the organism.

2.3.2.7 Growth on sole carbon source

2.3.2.7.1 Mannitol

The test organisms wee cultured into mannitol sugar medium (Appendix A). Positive result gave a pink color.

2.3.2.7.2 Rhamnose

The test organisms were cultured into rhamnose sugar medium (Appendix A).Production of a pink color, indicated acid production from rhamnose.

2.3.2.7.3 Arabinose

The test organisms were cultured into arabinose sugar medium (Appendix A). If the bacteria used the sugar and produced acid, the color of the medium changed to pink.

2.3.2.7.4 Sorbitol

The test organisms were cultured into sorbitol sugar medium (Appendix A). Positive result gave pink color indicating the production of acid from sorbitol.

2.3.2.7.5 Salicin

The test organisms were cultured into salicin medium (Appendix A). If the organism used salicin and produce acid, the color of the medium changed to pink.

2.3.3 Growth at 45°C

All the isolates were subjected to incubation temperature at 45°C. Inoculated T. S. A plates were examined for growth daily for up to 7 days.

2.3.4 Extraction and Analysis of Mycolic Acids

Sample of biomass from the test strains were degraded by acid methanolysis (Minnikin, 1988) as follows:

- Cell biomass (5 mg) was placed in bijou bottle.
- One ml of methanol/toluene/concentrated sulphuric acid (30:15: V/V) was added and incubated over night at 80°C.
- After cooling, the preparation was shaken with 1ml petroleum ether for 30 minutes.

- The supernatant which contained mycolic acids was transferred to a capped corning tube and evaporated to dryness at 37°C
- Then redissolved in 0.1ml petroleum ether. 5-10µL was spotted onto thin layer chromatographic TLC aluminum sheet (Merck).
- The sheet was run twice in solvent containing petroleum ether/diethyl ether (85:15;v/v).
- Then after dryness, the sheet was stained with 5% ethanolic molybdophosphoric acid and heated at 100°-150°C for 5-10 minute. Mycolic acid appeared as dark spots on a green back ground.

2.4 Anti –microbial susceptibility testing

The identified nocardial Isolates were tested for antibiotic sensitivity by modified Kirby Bauer disc diffusion technique (Kirby and Bauer, 1966). Four to five well- isolated colonies were suspended in 4ml peptone water, incubated for two hrs, then diluted with sterile normal saline until the density of suspension to be inoculated was equal to Mc Farland 0.5 STD tube. Two ml of diluted culture was poured and spread evenly on the surface of T. S. A. Excess fluid was aspirated and the plates were allowed to dry. Using forceps, antibiotic discs were placed on the agar. Gentle pressure was applied to the discs to ensure firm contact with the agar. Inoculated plates were left on the bench for

15 minutes, then incubated over night at 37°C. The antibiotic used were: Gentamicin (10µg), Amikacin(30µg), Tobramicin(10µg) and Erythromicin(15µg). Inhibition zone diameter was measured after the incubation period and the sensitivity results were interpretated according to National Committee for Clinical Laboratory Standards (NCCLS) published guidelines(1999).

2.5 Experimental induction of mastitis

Two lactating Nubian goats were selected for the induction of experimental mastitis. Two strains of *N. farcinica* (AB14, AB15) and a positive control (*N. farcinica* SD1800) were used. The two strains AB14 and AB15 had been isolated from the fence soil at the Department of Preventive Medicine. Faculty of Veterinary Medicine. U. of K.. This soil was previously contaminated with milk of goats experimentally infected with *N.farcinica.*

Due the fact that the colonies of noacardia were embedded in the agar and were difficult to suspend, a portion of the agar containing 2-3 colonies was aseptically cut and transferred into a tube containing sand beads, which was vortexed for 15 minutes to obtain a bacterial suspension. The viable bacterial counting method for the inoculum was done according to Miles Misra technique (Quinn *et al.,* 1999).

The left half of goat number (1) was used as negative control and the right half was infused with 1ml of *N. farcinica* (AB14) suspension containing $4.5x10^4$ CFU/ml. The left half of goat (2) was used as a positive control and was infused with 1ml of *N. farcinica* SD 1800 suspension containing $4x10^2$. The right half was injected with 1ml of *N. farcinica* suspension containing AB15 $8.8x10^8$ CFU/ml.

Before infusion, complete milking of the udder was performed. The surface of the udder and teats were washed and the teats orifices were carefully disinfected with 70% alcohol. The preparation of the inoclum, the cultural and microscopical examination of the milk samples post inoculation were all done under aseptic condition.

After inoculums infusion, the gland was massaged to distribute the inoculum, and it was not milked for 24 hours in order to allow the organism to establish itself. Daily rectal temperature, Rapid Mastitis Test and clinical observation were recorded for each experimentally infected goat.

2.5.1 Somatic cell count

The direct microscopic somatic cell count (DMSCC) was done by evenly spreading a measured volume of milk over a calibrated area of a microscopic slide, the film was stained and somatic cells with in a specified area of the film was counted (Packard *et al.,* 1992). The

count was then converted to cells per milliter (ml) by a factor which was determined by the magnification and area counted (Appendix B).

2.5.2 Milk samples

. Milk samples from each half were taken aseptically every two days. The udder was washed by a fresh solution of potassium permanganate and the teats were wiped with 70% alcohol and left it to dry. The first part of the milk was discarded, then about 10 ml of milk was placed in McCartney bottles and sent immediately to the lab for culturing.

2.5.3 Culturing

Tryptic soy agar and glucose yeast extract agar medium were used to culture milk samples

Each milk sample was centrifuged at 6200 rpm for 5 minutes and the precipitate was used for culture. Inoculated media were incubated aerobically at 37°C for 3-5 days and were examined daily for growth.

2.5.4 Rapid mastitis test (RMT)

The California (Rapid) mastitis test (CMT) was used for the detection of mastitis development.

About 3 ml of goat milk was poured into the shallow cups of RMT tray and then an equal volume of the test solution was squeezed into

the milk. The milk and the reagent were thoroughly mixed by gentle circular rotation of the tray which was held horizontally. After each test the tray was thoroughly rinsed with water

2.5.5 Treatment of infected goats

35 days post inoculation; the experimentally infected goats were treated with the broad spectrum intramammary preparation Neomastipra. Before infusion, teats were emptied, cleaned and disinfected. Treatment continued for 3 consecutive days.

Samples for somatic cell count and microscopic examination were taken during and after treatment.

After the withdrawal period of Neomastipra, the infected teats were further treated with gentamast for 3 successive days. Samples for investigation were taken as in the case of Neomastipra infusions.

CHAPTER THREE

RESULTS

3.1 Isolation Of nocardiae from soil

Eight soil samples from different sites of Sudan were used to isolate nocardiae. The numbers and types of nocardia like colonies isolated from the 8 sites were variable. Some plates showed a lot of colonies. Some plate showed 2 types of colonies whereas others revealed 3 types of colonies (Fig.1).

Colonies varied from rough to smooth, deeply embedded to superficially and easily detached. The colors were variable from creamy to orange (Table 2).

Nine nocardia isolates were recovered from the 8 soil samples examind.

The nine isolates were found Gram positive. They showed branched-cells, which fragment into pleomorphic, rod-shaped and coccoid elements(Fig.2). The strains were partial acid fast when stained with modified Ziel Neelsen stain.

Fig.1: The primary culture of *Nocardia spp*. on T.S.A.

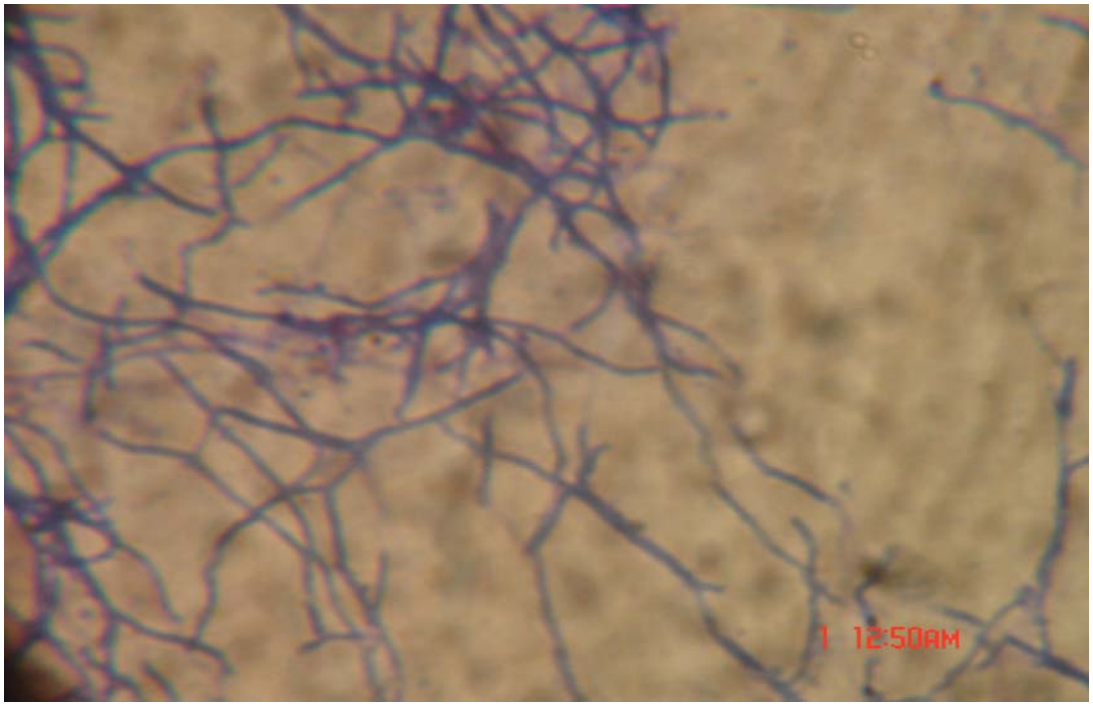


Fig 2: *N. farcinica* strain AB14. stain MZN. (X100)

Table 2: Colony characteristics of Nocardiae isolated from soil

Isolates	Rough	Smooth	Easily detached from agar	Embedded	***Color***			
					cream	Dark Cream	Oranges	Brown
SD2102	+	-	-	+	+	-	-	-
SD2103	+	-	+	-	-	-	+	-
SD2104	+	-	+	-	-	-	+	-
SD2105	+	-	-	+	-	-	+	-
SD2107	+	-	-	+	-	-	+	-
SD2108	+	-	-	+	+	-	-	-
SD2106	+	-	+	-	+	-	-	-
AB 14	+	-	-	+	+	-	-	-
AB 15	+	-	-	+	+	-	-	-

Table 3: Biochemical reactions of the Nocardia isolated from soil

Isolates	Tyrosin	Starch	Xanthin	Casein	Manitol	Soibitol	Rhamnose	Arabinose	Salicin	Urease	Catalse	My colic acid	Gro
2103	+	+	-	+	+	-	-	-	+	+	+	+	
2104	+	+	-	+	+	-	-	-	+	+	+	+	
2105	-	+	-	-	-	-	-	-	+	+	+	+	
2107	+	+	-	+	-	-	-		+	+	+	-	
2108	+	ND	-	+	-	-	+		+	+	+	-	
AB14	+	+	-	+	-	-	-	-	+	+	+	-	
AB15	+	+	-	+	-	-	-	-	+	+	+	+	

3. 2 Biochemical Tests

The result of the biochemical tests of the isolates is shown in Table 3.

3. 3 Cluster Analysis

According to the biochemical reaction of the isolates, three phenotypic clusters were obtained, these were as follows:

3. 3. 1 Cluster 1 (*N. brasilliensis*)

This cluster contained 2 isolates; SD2103 and SD2104. They were positive for casein, tyrosine (Fig:3), starch, urease, mannitol and salicin and were negative for xanthine, rhaminose, sorbitol and arbinose.

3. 3. 2 Cluster 2 (*N.asteroides*)

This cluster contained 1 isolate SD2105. It contained coccobacillary forms, it was negative for casein, tyrosine, xanthine and positive for starch, catalase, urease and salicin .

3. 3. 3 Cluster 3 (*N. f arcinica)*

Four isolates (SD2107, SD2108, AB14 and AB15) were identified in this cluster. They were positive for casein, tyrosine, salicin and starch and were negative for xanthine, manitol, sorbitol and rhamnose.

Fig.3 Growth of *N. brasilliensis* SD2103 on tyrosin medium

Two isolates (SD2102, SD2106) were phenotypically and microscopically were typical to Nocardia but the biochemical tests were not done for them.

3.4 Mycolic acid analysis

All isolates, except three (SD2107, SD2108 and AB14), revealed mycolic acids on TLC plates (Fig.4). The positive control (N36) revealed single major spot characteristic for mycolic acids of nocardiae. The negative control revealed no my colic acids on TLC.

.3.5 Antibiotic sensitivity testing

Table (4) shows the results of sensitivity of nocardiae isolated from soil to various antibiotics.

It is clear that gentamycin (10 μg), tobramycin (10 μg) and amikacin (30μg) were the drugs which showed wider inhibition zones, But erythromycin (15 μg) inhibited in vitro all test isolates except two (SD2105 was moderate inhibition and AB15 was resistant.

3.6 Experimental induction of mastitis in Nubian goats

3.6.1 Goat 1

One half of the udder was inoculated with *N. farcinica* strain AB14. After the first 48 hours post inoculation, no abnormalities were

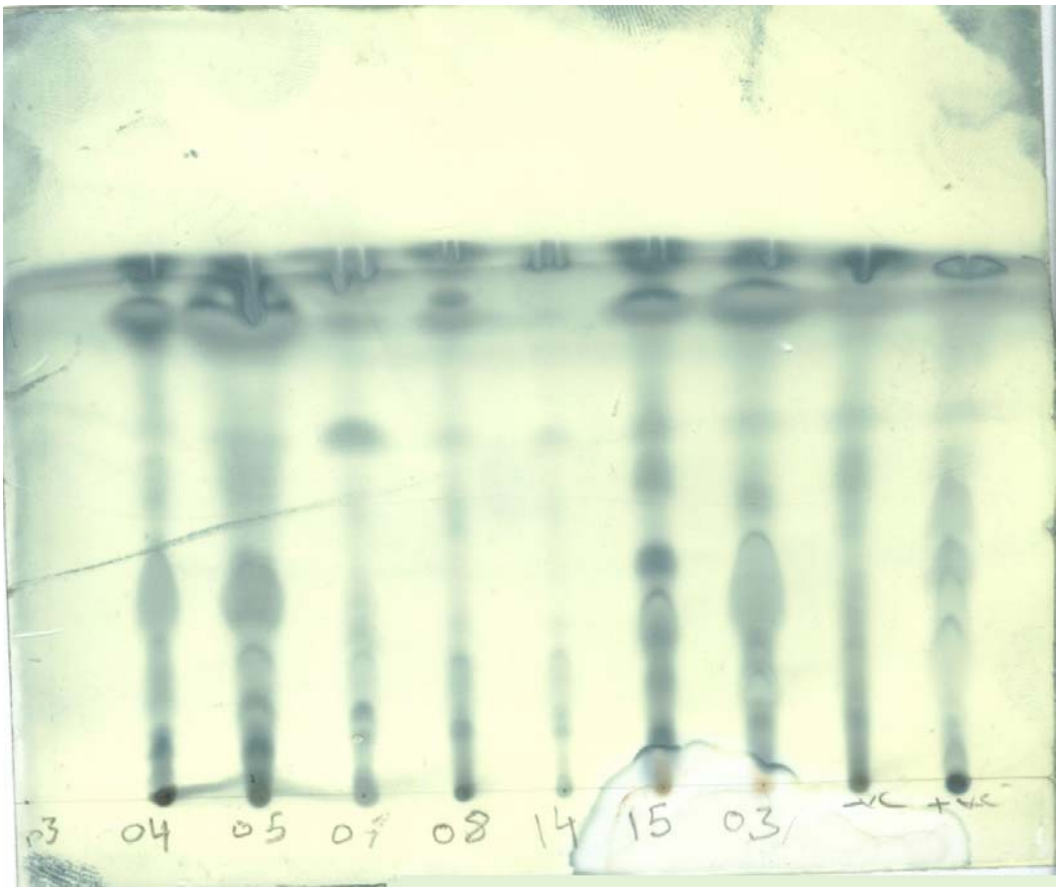


Fig 4. Thin Layer chromatographic analysis of my colic acids methylesters of some *Nocardia spp.* Isolated from Sudanese soil in comparison to positive control N36 and negative control. Staph. aerus.

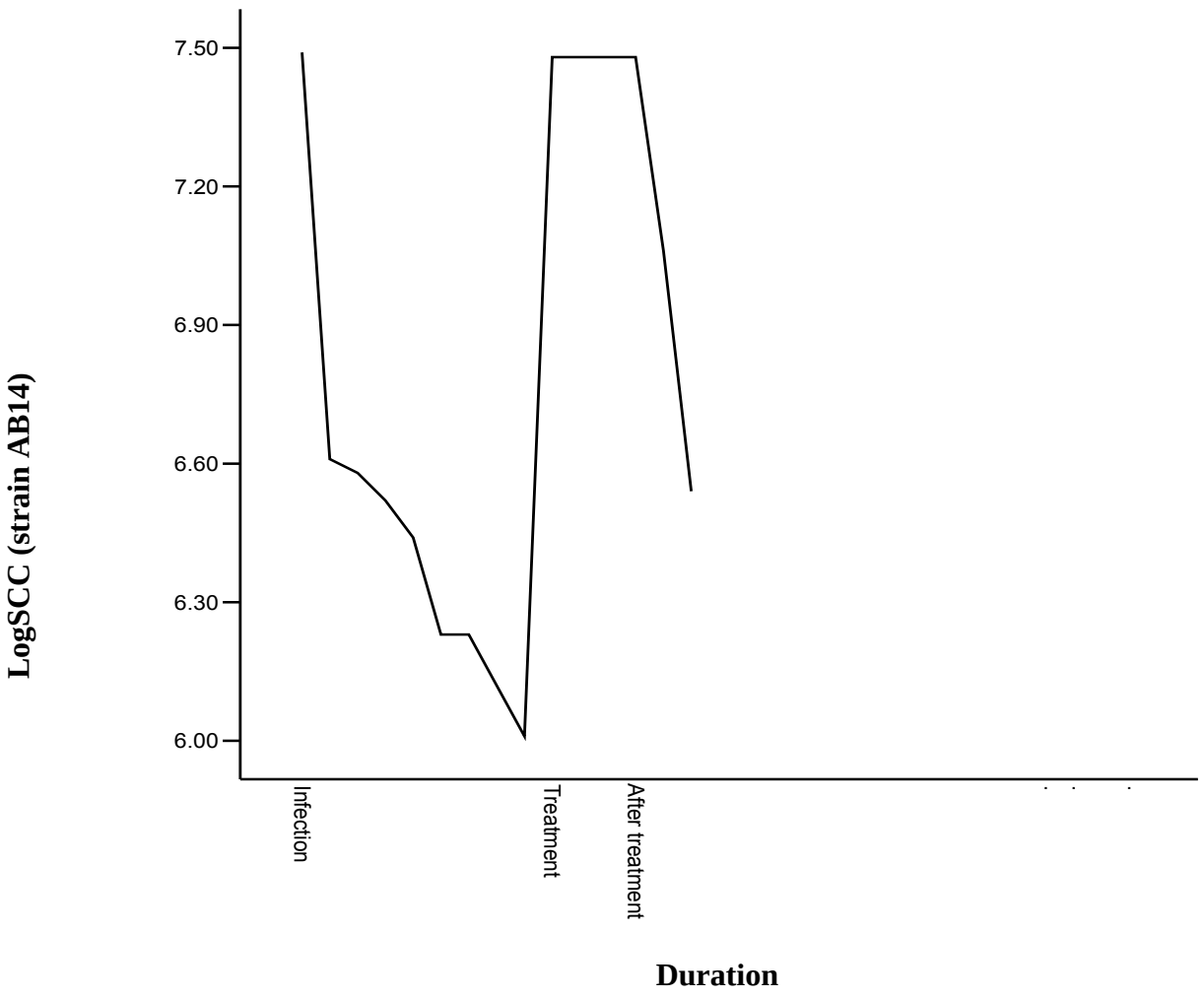
04 = SD 2104 - ve. Negative control

05 = SD2105 + ve. Positive control

Table 4: Results of Antibiotic Sensitivity test

Nocardia isolate	**Gentamycin**	Tabramycin 10μg	Amikacin Ak 30 μg	Erythro mycin 15 μg
SD2103	sensitive	sensitive	sensitive	sensitive
2104	sensitive	sensitive	sensitive	sensitive
2105	sensitive	sensitive	sensitive	intermediate
SD 2107	sensitive	sensitive	sensitive	sensitive
SD2108	sensitive	sensitive	sensitive	sensitive
AB15	sensitive	sensitive	sensitive	resistant

Fig 5 Somatic Cell Count in goat I (right udder)



noticed in thise goat.The udder and milk secretions were normal. Milk samples in day 3 post inoculation revealed a high leukocytes count and a positive rapid mastitis test (RMT) with no change in milk color or udder condition but decrease in milk yield was detected. Appetite of the goats and body temperature remained normal. On the day 8 post inoculation there was high leukocyte count, smears from mammary secretion revealed gram-positive and partial acid fast branching filaments when stained with MZN. The organism was re-isolated and the excretion of organism in the milk was intermittent. By the day 12 post inoculation the milk of infected udder become yellowish and the leukocyte count persisted high.

Thirty five days post infection, the affected half of each goat was treated with intramammary infusion (Neomastipra). The mammary secretion was collected and examined bacteriologically prior treatment. The milk appeared normal, while smears and cultures on T.S.A media revealed the organism in milk stream. 24 hrs after treatment, mammary secretion of the affected half changed to yelowish-viscous, clotted, oily secretion with high leukocyte count (uncounted).

During the course of treatment the leukocyte count remained uncountable (Fig. 5).

3.6.2 Goat II

This goat was inculcated with two isolates of *N. farcinica.* The right half of the udder was inoculated with *N. farcinica* isolate AB15 and the left half was inoculated with *N. farcinica* 1800 as positive control.

After 48 hours post inoculation no abnormalities were seen in this goat. The udder and milk secretion were normal. By the third day post inoculation there was a high leukocyte count and R.M.T was positive for both halves. Milk yield decreased, appetite and the body temperature remained normal, Leukocyte count of the right half, inoculated with AB15, was uncountable(fig: 6) and the left half that inoculated with SD 1800 (Positive control) was high but it was countable (Fig. 7).

Smears from mammary secretion revealed Gram positive and partial acid fast branching filaments in case of SD 1800 and coccoid forms in case of AB15 when stained with MZN. The organisms were recovered in pure culture.

The two halves were treated with intramammary antibiotic infusion (Neomastipra). The mammary secretion was collected before treatment and examined bacteriologically. The milk was normal, stained smears and culturing on T.S.A media revealed the organism.

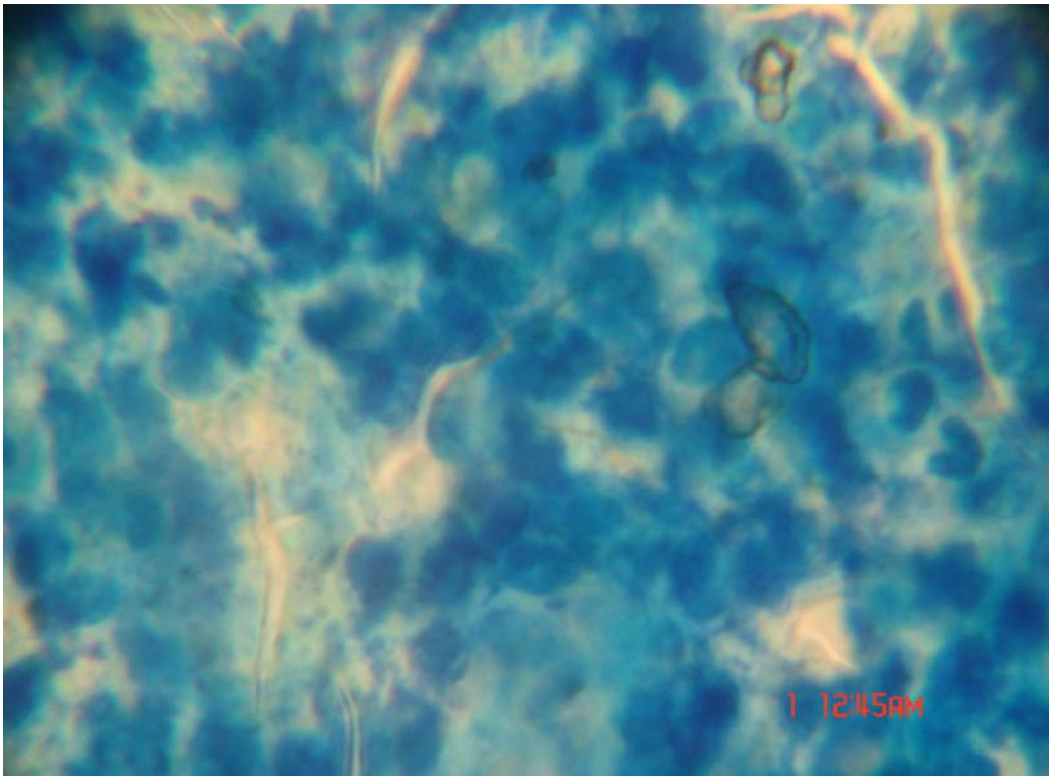


Fig. 6. SCC. Stain Newman stain. (X100).

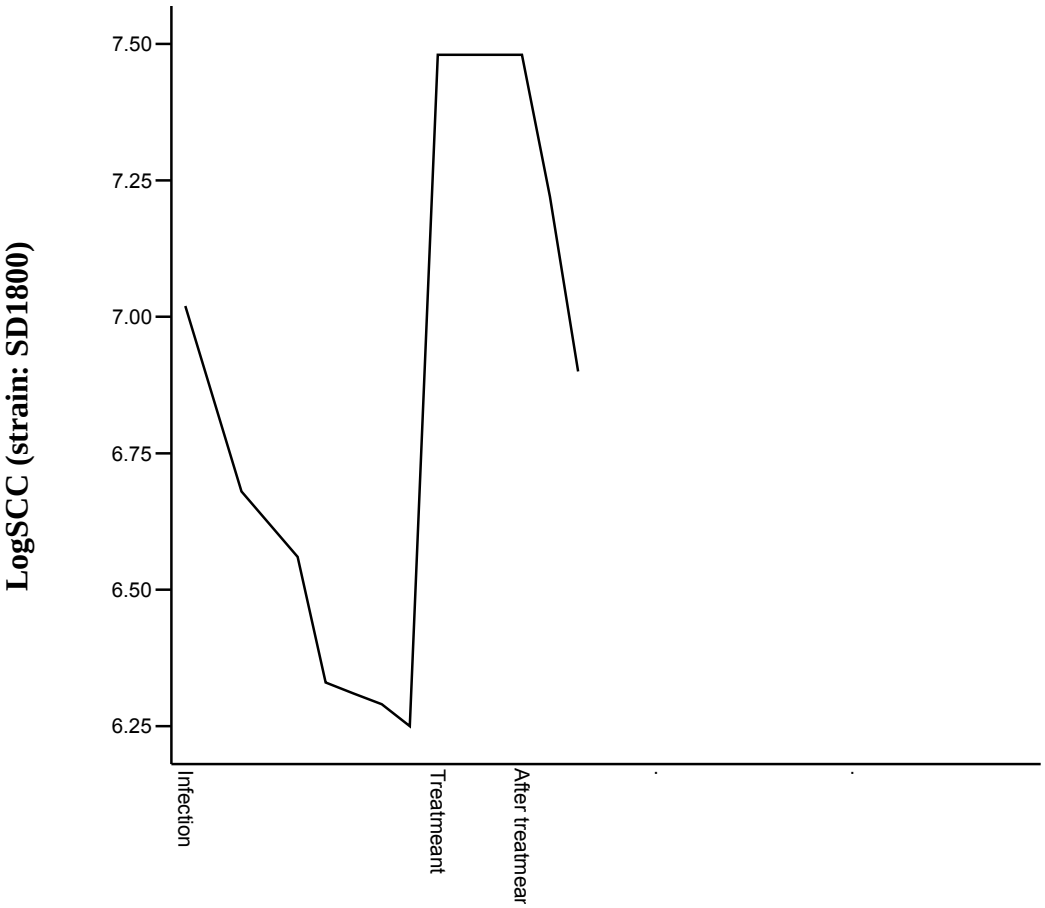
Three days after the last dose of treatment the leukocyte count remained high (uncounted) and milk color was yellowish with clots.

After the withdrawal period of the drug (4days) the milk secretion, color and consistency returned to normal and with decreased leukocytes count (Fig.5). Cultural and microscobical examination of milk samples from infected half during and after treatment with Neomastipra were positive for nocardia.

Seventy two hours after the drawl period starting treatment with Gentamast. The course of Gentamast treatment the leukocyte count was high (uncountable), the milk color was whitish with oil secretion and diarrhea.

Twenty four hrs after last dose of treatment leukocyte count persisted high (uncountable) and the milk color whitish with diarrhea continued. After drawl period there is high leukocyte count (un countable) and milk color is whitish.

Seventy two hrs after last dose of treatment the milk secretion decreased sharply and milking the affected half was done by teat siphoning and the color was whitish. The Cultural and microscopic examination of milk sample from infected half were positive for Nocardia.


LogSCC (strain: SD1800)
7.50
7.25
7.00
6.75
6.50
6.25
Infection
Treatmeant
After treatmear

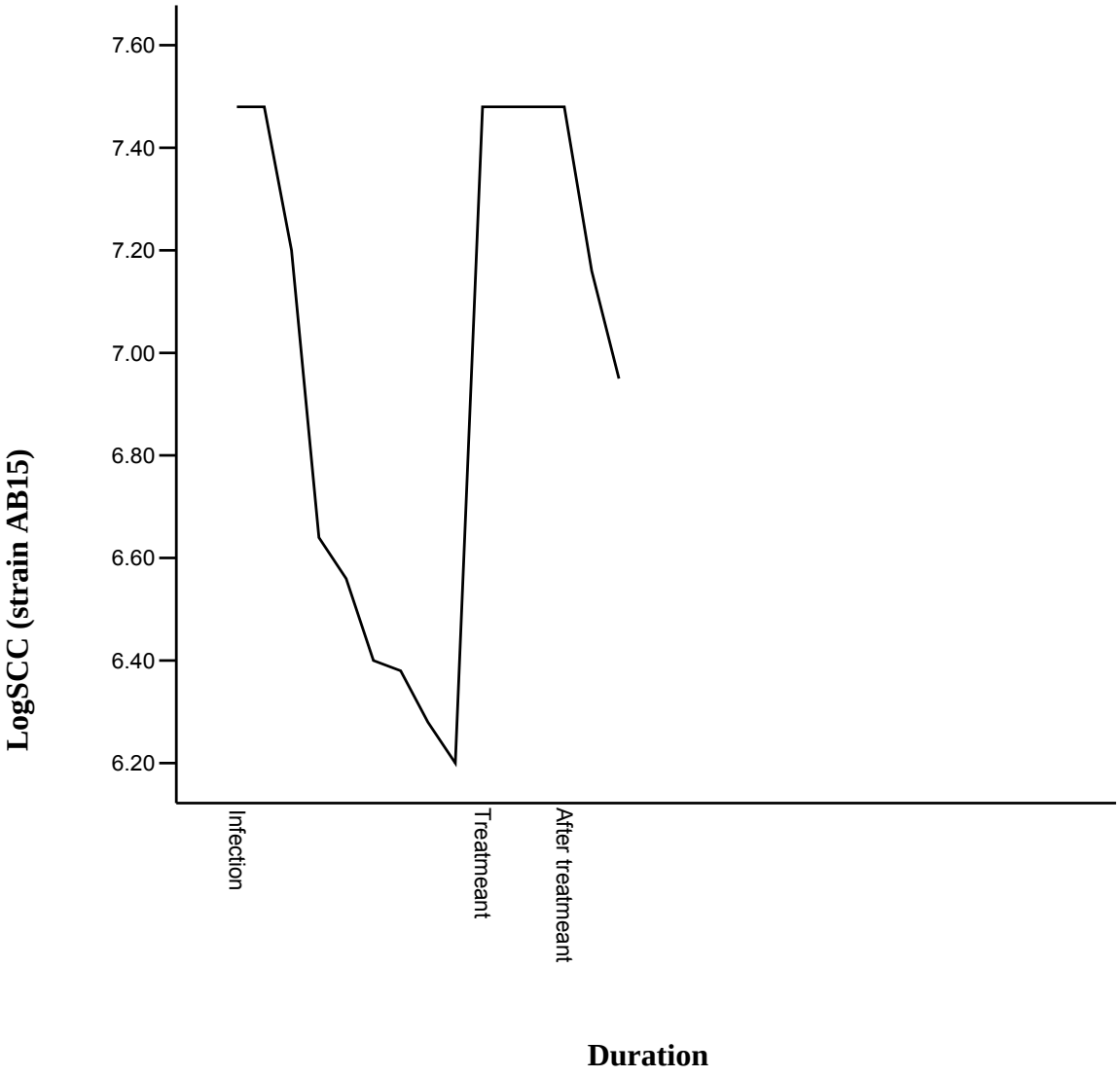
One day after treatment, mammary secretion of the two halves changed to yellowish, viscous clotted, oily secretion with high leukocyte count. Two days post treatment the leukocyte count remained high and the milk color was yellowish, viscous with oily clots.

After the last dose of treatment the leukocytescount was still high and the color of the milk was yellowish viscous with clots and oily secretion.

After the withdrawl period of the drug (Neomastipra) the milk from the two halves appeared normal but stained smears and cultures on T.S.A media revealed the organisms. 72 hours after the with drawl- period of Neomastipra treatment with Gentamast was attempted.

During treatment with Gentamast there was high leukocyte count (uncountable) while the milk color appeared normal.

Twenty four and 48 hours after the last dose of treatment also the leukocytes were uncountable in case of strain SD 1800 but it was countable in case of the strain AB 15 (fig:8). The cultural and microscopic examination of milk samples from two halves were positive for Nocardia.


LogSCC (strain AB15)
7.60
7.40
7.20
7.00
6.80
6.60
6.40
6.20
Infection
Treatmeant
After treatmeant
Duration

CHAPTER FOUR

DISCUSSION

The present study was performed to isolate and identify *Nocardia spp*. from soil, to study the persistence of *Nocardia spp*. in goat bedding and to experimentally induce mastitis in goats with these soil isolates compared *to* infection induced by clinical isolates.

Nocardiae are widely distrubuted and are abundant in soil and in other environment (Goodfellow, 1998). Many studies have reported the isolation of nocardiae from soil (Orchard and Goodfellow, 1980, Volera *et al. 1980 and Maldonado et al*., 2000). *N. asteroides* was *isolated* from soil of Kuwait (Khan *et al.,* 1997). The isolation of nocardiae was also reported from the Sudan (Sid Ahmed, 2001).

In this study, Tryptic Soy Agar supplemented with tetracycline (5µg/ml), nystatine (50µg/ml) and NaCl 5% was found to be a good medium for isolation of Nocardia spp. from soil and this result authenticated the work of Sid Ahmed (2001) who used Tryptic Soy Agar supplemented with tetracycline, nystatin and also consistent with the work of Gamal Eldin (2003) who isolated Nocardia from milk on Tryptic soy Agar supplemented with NaCl.

Other media notably Lowen Stein-Jenson (Hamid, *et al.,* 1998), brain heart infusion agar and Sabouroud Detrose Agar (Goodfellow, 1998) were also used for the isolation of nocardiae from clinical samples.

Identification of nocardiae to species level was reported to be difficult due to dearth of suitable phenotypic tests (Goodfellow, 1998), nevertheless, in this study combining conventional methods with mycolic acid analysis allowed the identification of the isolated nocardiae. Similar observations were reported by Hamid (1998) and Gamal Edin (2003) who experienced the difficulty of species identification.The findings in the present study, which were based on phenotypic characters mycolic acid analysis need to be confirmed by using appropriate molecular techniques such as the *16s* DNA analysis which had been widely used for accurate identification (Hamid *et al.,* 2001, Maldonado *et al.,* 2004).

In the present study, most of the isolated spp. contained mycolic acid and regarding their phenotypic characteristic, microscopic finding and result of biochemical test, they were identified as nocardia. Three isolates were found to possess no mycolic acid although they were phenotypically similar to nocardiae.

Antibiotic sensitivity of the isolates showed that the majority of the isolated nocardiae were highly sensitive to gentamycin (10 µg/mL), amikacin (30 µg/mL), tobramycin (10 µg/mL) and erythromycin. These results are partially consistent with the findings of Nassal (1967), Chen

(1993), Hamid et al., (2001) and Gamal Eldin (2003) who reported the sensitivity of nocardiae to gentamycin and erythromycin. This finding agreed with the result of Kiska (2002) who noticed that most nocardiae were sensitive to gentamycin and amikacin. Contrary to his result most spp in the present study were found sensitive to tobramycin and erythromycin. Two isolates were found of intermediate resistance to erythromycin.

Experimentally, *N. farcinca* strain ABI4, which was isolated from soil, induced mastitis in goat 1, which resembled the natural infections. Clinical signs indicative of mastitis were not detected in this goat but analysis of milk samples revealed high leukocyte count, in addition stained smears and cultures of milk on TSA were positive for nocardiae. These results were in accordance with that of Gamal Eldin (2003) who isolated nocardia from milk of clinically normal goat. The findings of the experimentally induced mastitis disagreed with Dafalla and Gharib (1958), Megid, *et al.* (1990) and Hillermark (1960) who reported granulomatous lesions associated with nocardial mastitis in goats. Results of this study are also inconsistent with those of Ibrahim (1968) who observed watery secretion from affected glands and Pier (1961) who reported progressive fibrosis with enlargement of mammary lymph nodes. Results are also contrary to these of Gamal Eldin (2003) who reported an acute mastitis with severe granulomatous lesions in experimental goat that was inoculated by *N. farcinica*.

Excretion of *Nocardia. spp* in milk of infected half was established in all cultured samples. A Similar finding was noticed by Wendt (1969) and Gamal Eldin (2003) who intermittently isolated the organism from experimentally infected goats. In this study the mild mastitis induced by nocardiae isolated from soil might be partially explained by the fact that soil isolates got adapted to saprophytic habitat and might have lost some of its virulence factors. In the present study anorexia and fever were not observed in the two experimentally infected goats. These observations were similar to the finding of Gamal Eldin (2003). However, Megid *et al.* (1990), observed anorexia and fever in goats.

Excretion of nocardia in mammary secretion and high leukocytes count were observed during and after treatment of the affected half with Neomastipra. This means that the treatment did not reduce or eradicate the organism from the udder. Similar findings were reported by Gamal Edin (2003). Treatment with gentamast also failed to eradicate the organism from the udder. Ahmed (2003) who recorded that gentamast had little effect against *N. farcinica* in vivo in cattle but growth of organism had been inhibited in vitro. .

The high leukocyte count during and after treatment recorded in the present study might be due to dead bacteria or the effect of antibiotic. Dead cells may release more antigens into the udder, which in turn might

have resulted in increased inflammatory response as indicated by WBC in milk secretion.

In the current study, 72 hrs after last dose of treatment the mammary secretion was scanty in Goat I which was inoculated with *N. farcinca* B14. These results agreed with the findings of Ibrahim (1968) who reported the complete absence of mammary secretion.

The left half (negative control) in goat 1 developed no infection and this fact proved that transmission of infection from an infected half to another did not occur. This observation was analogous to that of Gamel Eldin (2003) and Ahmed (2003).

Goat 2 that was inoculated with *N. farcinica* SD1800 (positive control) and *N. farcinica* AB15 developed mild mastitis associated with high leucocyte count. The mild nature of infection induced by *N. farcinica* SD1800 may be due to the reduction of pathogenicity of the bacterium as it was kept under laboratory conditions since it was first isolated in 1999 (Hamid *et al*., 2005). *N. farcinica* AB15 which was isolated from fence soil also induced mild mastitis.

Treatment with Neomastipra failed to eliminate the infection or eradicate the organisms from the udder and likewise did gentamicin although in both cases there were no visible clinical signs or lesions. This result agreed with Radostitis *et al*., (2000) who reported that the disease does not respond well to treatment.

CONCLUSIONS

The present study concluded that:

1. *Nocardiae* are readily available in soil samples.

2. Tryptic Soy Agar medium with combination of 5µg /ml tetracycline., 50µg /ml nystatine and 5% NaCL was found to be a suitable medium for isolation of *nocardiae* from soil.

3. Experimental infection with soil isolates of *N*. *farcinica* produced mild mastitis in goats.

4. *N. farcinica* which was found sensitive in vitro to many antibiotics, in vivo it is resistant to them (mastitis treatment).

RECOMMENDATIONS

The present study recommended for further research:

1. Studies are needed to isolate nocardiae and other saprophytic actinomycetes from soil in order to establishe ecological database of the organisms.
2. Molecular techniques should be implemented for accurate identification of nocardiae to strain level.
3. More investigations are needed in order to establish in vivo treatment of nocardia infections.
4. Further investigations are needed to know the effect of disinfectants and fence hygienic systems on Nocardia spp.

REFERENCES

Abu-Samra, Mukhtar T. ; Elsanousi, S. M.; Abd Alla, M. A. ; Gameel, A. A. ; Abd Elaziz , M. ; Abbas, B. ; Ibrahim, K. E. E. and Idris, S. O. (1988).Studies of gangrenous mastitis in goats. Cornell. Vet **78**: 281 – 300.

Ahmed, A.O. (2003).Bovine mastitis etiology, clinical aspects and treatment with special reference to actinomycetes infection. Ph. D. Thesis, University of Khartoum.

Ajello, G.; Brown, J. ; Mahgoub, E. L. and Ajello, L. (1979). Anote on the isolation of pathogenic aerobic actinomycetes from Sudanese soils. Current Microbiol. **2**: 25 – 26.

Anon (1981), Animal Bulletin of Animal Resources Statistics, Ministry of Agriculture and Irrigation, Sudan. Pp.29 – 34.

Anon, (1987). Bovine mastitis: Definition and guidelines for diagnosis. Bulletin of the International dairy Federation, No. 211.

Barrow, G. I. and Feltham, R. K. A. (1993). Cowan and steel's Manual for the identification of medical bacteria 3rd ed. Cambridge University press.

Bartlett, P. C.; Miller, G. Y.; Lance, S. E. and Heider, L. E. (1992). Environmental and managerial determinant of somatic cell counts and clinical mastitis incidence in Ohio dairy herds. Prev. Vet. Med. 14 (3-4): 195-207.

Blood, D. C.; Radostitis, O. M.andHanderson, J. A.(1983).Veterinary medicine. 6th edition, Bailliere Tindal, London, Pp. 451 – 493.

Bramley, A. J. (1975). Infection of the udder with coagulase- negative micrococci and corynebacterium bovis. In: Proc. Sem. Mastitis Controls, Int. Dairy Fed. Reading England, Pp. 377.

Carter, G. R. (1979). Diagnostic Procedure in veterinary Bacteriology and Mycology. (Charles, Thomas) U. S. A. P. 396.

Carter, G. R. (1996). Diagnostic Procedures in Veterinary Bacteriology and Mycology. 7th ed. Philadelphia, Charless, C. Thomas. Publishers, U. S. A.

Chen, S.C. and Wang, P. C. (1993). In vitro activity of antimicrobial agents against Nocardia asteroides. Journal of fish diseases. **16**: 269 – 272.

Conti-Diaz, I. A. ; Gezuele, E. ; Civila, E. and Mackinnon, J. E. (1971). Termotoleranciax accion patogena de cepas de Nocardia asteroides aislades de fuentes naturales. Revista Uvuguaya de patolgia Clinica x Microbiologia, **9** : 232 – 241.

Dafaalla, E. N. and Gharib, H. M. (1958). A study of mastitis on goats caused by *Nocardia asteroides*. Brit. Vet. J. 114: 143 – 145.

Devendara, C. and Maclery, B. G. eds (1983). Goats and sheep production in the tropic. Pp. 74 – 83.

Devendra , C. ; Lindley, E. P. ; Bahattacharya, P.; aulfernandez-Baca; Warner ; James ; Mann , I. ; Moule, G. R. and Baker, P. R**.** (1977). Animal Husbandry 18th ed. London. Pp. 505 - 536.

Devendra, C. (1966). The importance of goats in Malaysia. Z. Tierzucht.ZuchtBio., **83**: 72-9 (in English).

Devendra, C. and Burns, M. (1983). Goat production in the tropics. Tech. Commun. Comw. Bur. Anim. Breed. Genet, CAB: Farnham Royal, UK.

Dorsey William Burner and James Haward Gillespie. (1973). Actinomycetaceae. In Dorsey (6th ed). Hagan's . Infectious disease of domestic animal. 6th ed. Cornell University Press. Pp. 467 - 471.

Dwight C. Hirsh and Yuan Chung Zee (1999). Pathogenic Actinomycetes In Dwight C. Hirsh. Veterinary Microbiology. 6 Black well science Pp.250 – 255.

Erskine, R. J. ; Eberhart, R. J. ; Hutchinson, L. J. ; Spencer, S. B. and Campbell, M. A. (1988). Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. J. Am. Vet. Med. Assoc.**192**: 761 – 765.

Ferns, L. ; Dohoo,I.R. and Donald, A. (1991). Acase control study of *Nocardia* mastitis in Nova Socia dairy herds Candidian. Vet.J. **32**: 673-677.

Gamal Edin, O. A. (2003). Studies on caprine mastitis caused by nocardiae actinomycetes; M. V. Sc. Thesis, University of Khartoum.

Goodfellow, M. and Lechavalier, M. A. (1989). Genus *Nocardia Trevision* 1889, 9AL, In Bergery's Manual of Systematic Bacteriology, Vol**. 4.** Williams, S. T., Williams and Wilkins, Baltimore, 2350 – 2361.

Goodfellow, M. (1998). Nocardia and related genera. P. p. 463 – 489. In, Aballows and B. I. Duerden (ed). Topley and Willson's Microbiology and microbial infection. 9th edition Ool. 1, Systemic Bacteriolog . Edward Arriold. London.

Gordon, R. E. and Hagan, W. A. (1936). A study of some acid fast actinomycetes from soil with special reference to pathogenicity to animal. J. of Infect. Dis. 200 – 2006.

Hamid, M. E. ; Elsanousi, S. M. ; Minnikin, D. E. and Goodfellow, M. (1998). Isolation of *Nocardia farcinica* from Zebu cattle suffering from mastitis in Sudan. J. Vet. Sci. Anim. Hsb. **37**: 66– 71.

Hamid, M. E. ; Gamal Eldin, O. A. ; El Hussein, H. A. ; Ibrhim, K. E. Abdel Salam, E. B. (2005). First report of a granulomatous nodular mastitis in a Saanen goats caused by *Nocardia farcinica*. Sud. J. Vet. Res. (in Press).

Hamid, M. E. and Goodfellow, M. (1997).Invitro antimicrobial susceptibility of bovine farcy organisms. Rev. Ele. Med. Vet. Pays Trop. **50**:1–5.

Hamid, M.E. ; Maldonado, L. ;Sharf Eldin, G. ; Mohammed, M. ; Saeed, N. and Goodfellow, M. (2001). *Nocardia africana* sp.

Nov., a new pathogen isolated from patients with pulmonary infections. J. Clin. Microbiol. **39**: 625 – 630.

Harmon, R. J. and Langlois, B. E. (1986). Prevalence of the minor mastitis pathogens associated with somatic cell counts. Proc. 25th. annual Meeting of National Mastitis Council, Inc., Arlington, VA, PP. 11.

Hillermark, K. (1960). *Nocardia asteroide* as cause of bovine mastitis. Acta Vet. Scand. **1**: 181 – 293.

Ibrahim, A. E. (1968). A preliminary survey of the etiology of mastitis among goats and sheep around Khartoum province; M. V. Sc. Thesis, University of Khartoum.

Ibrahim. A. E. (1962). Caprine mastitis caused by *mycblasma, Nocardia* and *corynebactrium pseudotuberculosis*. S. J. Vet. Sci. Anim. Husb**.** **9**: 31– 35.

Khan, Z. U, Neil, L. ; Chandly, R. ;Chung, I. D. ; Al – Sayer, H. ; Provost, F. and Boiron, P. (1997). *Nocardia asteroides* in the soil of Kuwait. Springer link – Mycopathologia. **137**: 159 – 163.

Kirby, W. M. M., Bauer, A. W., Sherris, J. C., Turck, M., (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**: 493 – 496.

Kirk, J. J. (1984). Somatic cells in milk. Current Concepts. Compend. Contin. Educ. Prac. Vet. 6: S237 – S243.

Kirpensterijn, J. and Fingland, R. B. (1992). Cutaneous actinomycosis and nocardiosis in dogs, 48 cases (1980 – 1990). J. Amer. Vet. Med. Asso. **201**: 917 – 920.

Kiska, Deanna., Karen Hicks. And Pettit, David J. (2002).Identification of medically relevant *Nocardia* species with an abbreviated battery of tests. J.Clin. Microbiol. **40**: 1346 – 1351.

Kumar, R. Mohapatra, L.N. (1968). Studies on aerobic actinomycetes isolated from soil. Isolation and identification of strains. Sabouraudia, **6**: 140- -146.

Kurp, P. V. ; Randhawa, H. S. and Mishra, S. K. (1976)Use of paraffin bait technique in the isolation of *Nocardia asteroides* from sputum.Mycopathologia et Mycologia Applicata, **40**: 363– 365.

Kurp, P. V. ; Randhawa, H. S. and Sandhu, R. S. (1968). Survey of *Nocardia asteroides*, *N. caviae* and *N. brasilliensis* occurring in soil in India. Sabouraudia, **6**: 260 – 266.

Kurp, P. V. and Sandhu, R. S. (1965). Isolation of *Nocardia caviae* from soil and its pathogenicity for laboratory animals. J. of Bacteriol. **90**: 822 – 823.

Lerner, P. I. and Baum, G. L. (1974). Antimicrobial susceptibility of *Nocardia* species. Antimicrobial agent and chemotherapy. **4**:85 – 93.

Lynch, J. A. (1990). Nocardial mastitis selective medium. Canad. Vet. J. **31**: 417.

Maldonado, L.; Hookey, V. J. ; Ward, C. A. and Goodfellow, M. (2000). The *Nocardiasalmonicida* clade, including descriptions of *Nocardia cummidelens* sp*. Nocardia fluminea* sp. *nov and Nocardia soli* sp*.*

nove. Antonie Van Leeuwenhoek, **78**: 367–377.2001 Kluwer Academic publisher. Printed in the Netherlands.

Maldonado, L. A.; Hamid, M. E. ; Gamal Eldin. O. A. andGoodfellow, M. (2004). *Nocardia farcinica* – a significant cause of mastitis in Sudan. S. Afr. Vet. Ass.(2004) 75 (3): 147 – 149.

Mc Clung, N. M. (1960). Isolation of *Nocardia asteroides* from soil. Mycologia, **52**: 154–156.

McNeil, M. M. ; Barown, J. M.; *et al*. (1994).The medically important aerobic actinomycetes : epidemiology and microbiology. Clin. Microbiol. Rev. **7**: 357 – 412.

Megid, J.; Muller, E. E. ; Feritas, J. C. and de\ Vlotti, N. M. A.; Bracarense, A. P. F. R. L. ; Casta, E. O. ; da\ Countinho, S. D. (1990). Nocardia asteroides mastitis in goats. Arquivo Brasileiro de medicina Veterinaria Zoottecnia. **42**: 545 – 547.

Minnkin, D.E. (1988). Isolation and purification of mycobacterial wal lipids. In: Bacterial Cell Surface Techniques, Pp. 125 – 135 Edited by I. C. Hancok and I. C. Poxton. Winchester, John Wiley and Sons.

Mishra, S. K. and Randhawa, H. S. (1969) Application of paraffin bait technique to the isolation of *Nocardia asteriodes* from Clinical Specimens. Appl. Microbiol., **18:** 686-687.

Morin, D. E. and Hurley, W. L. (1993). Ecnomic analysis of mastitis monitoring and control program in four dairy herds. J. Am. Vet. Med.

Nassal, J. (1967). Acute *Nocardia* mastitis in a cow. Dt. Tieraztl. Wschr. **74**: 434 – 437.

National Committee for Clinical Laboratory Standard.,(1999). Performance standards for antimicrobial susceptibility testing. 9th information supply. National committee for Clinical Laboratory Standards. Wayne, P. A.

Nesbaken, T. (1976). The cell content in milk of goats. Nordisk veternaer

Orchard, V. A. and Goodfellow, M. (1974). The selective isolation of *Nocardia* from soil using antibiotics. J. Gen. Microbiol. **85**: 160-2.

Orchard, V. A. and Goodfellow, M. Numerical classification of some named strains of Nocardia asteroids and related isolates from soil.. J. Gen. Microbiol. **118**: 295 – 312.

Pachard, V. s.; Tatini, S. ; Fugua, R. ; Heady, J. and Gilman, C. (1992). Direct microscopic methods for bacteria or somatic cell In: Marshall, R. T. (ed). Standard methods for the examination

of dairy products, 16th ed. American Public Health Association, USA, Washington D.C., Pp. 309–325.

Parsad, L. N; Gupta, p. p. and Singh, N. (1985). Experimintal arginini mastitis in goat. Aust. Vet. J**. 62***:*341 – 342.

Patrick R. Murray; Ellen Jo Baron; James H. Jorgensen; Michael A. Pfaller and Robert H. Yolken. (2003). Manual of clinical microbiology. 8th ed. ASM press Washington, D. C. Pp. 502 – 524.

Payne, W. J. A. (1990). An Introduction to animal husbandry in the tropics. 4th ed. ELBS with Longman UK Lted:463 - 483.

Pier, A. C. ; Mejia, M. J. and Willers, E. H. (1961). *Nocardia asteroides* as mammary pathogen of cattle. A mer. J. Vet. Res. **22:** 502 – 517.

Quinn, P. J.; Carter, M. E. ; Markey, B. K. and Carter, G. R. (1999). Mastitis. In: Quinn, P. J. (ed) Clinical veterinary microbiology. Mosby London Pp. 327 – 344.

Quinn, P. J.; Markey, B.K. ; Carter, M.E. ; Donnelly, W. J. and Leonard, F.C. (2002). Veterinary Microbiology and Microbial Disease. 1st ed., Blackwell Science: 465 – 475.

Quinn, P. J.; Carter, M. E.; Markey, B. K. and Carter, G. R. (1994). Mastitis. In: Quinn, P. J. (ed) Clinical veterinary microbiology. Wolfe, Baltimore, Pp. 327 – 344.

Rodostitis, O. M.; Blood, D. C. and Henderson, J. A. (1983). Veterinary medicine. 6th ed. London, Bailliere Tindall. Pp. 363 – 384.

Rodostitis, O. M.; Leslie, K. E. and Fetrow, J. (1996). Mastitis control in dairy herds. In: Rodostitis, O. M., Leslie, K.E. and Fetrow, J. (eds). Herd Health: Food Animal Production Medicine. 2nd ed. Philadelphia: W. B. Saunders Company. Pp. 229 – 276.

Rodostitis, O. M.; Gay, C. C. ; Blood, D. C. and Hinchcliff, K. W. (2000). In: Veterinary medicine. A Teext book of the disease of cattle, Sheep, pigs, Goats and Horses. Mastitis 9th ed .W. B. Saunders. Pp. 603 – 700.

Savalia, C. V. and Kher, H. N. Incidence of nocardial mastitis in a coro. Cheiron. **19**: 328.

Schconder woerd, M. ; Mc Fadzen, L.L. ; Mannimen, K.I. and Ollis, G.W. (19990). Culturing of bulk tank milk for the presence of *Nocardia spp*. Canada. Vet. J. **31**: 435 – 454.

Shigidi, M. T. and Mammoun, E. (1981). Isolation of *Nocardia asteroids* from cattle with mastitis in Sudan. Bull. Anim. Hth. Prod. Afric. **29**: 276 –278.

Sid Ahmed, A. (2001). Isolation and identification of pathogenic nocardiae J. **32:** 673–677.from soil samples in Khartoum state; M. V. Sc. Thesis, University of Khartoum.

Siddique, I. H.; Hafeez, M. and Gbadamosi, S. G. (1988). Screening for sub-clinical mastitis in goats. Testng the tests. Veterinary medicine. **83**: 87– 88.

Tripathi. B. N. and Chattopadhyay, S.K. (1993). Caprine mastitis clinical morphological and epidemiological findings in spontaneous occurring cases in Indian goats. Inter. J. Anim. Sci. **8**: 107 – 111.

Valerie, A. ; Orchard, and Goodfellow, M. (1974). The selective isolation of *Nocardia* from soil using antibiotics. J. Gen. Microbiol. **85**: 60 -162.

Vandime, A, ; Pageaux, G.P. ; Bismuth, M. ; Fabre, J. M. ; Mamergue, J. ; Perez, C. ; Makeieff,M. ;Mourad, G. and Larrey, D. (2001). Nocardia revealed by thyroid abcess in a live kidned transpiant recipient. Transpl. Int. 2001, **14:** 202 – 204.

Wakeen, A. A.; and ElTayeb, A. (1962). A survey of the causal organisms of chronic mastitis in a dairy herds and it's treatment with oxytetracycline. Sudan. J. Vet. Sci. Anim. Husb,., **3**: 28 – 32.

Wendit, K.; Pospisil, M. and Funchs, H. W. (1969). Endemic mastitis caused by *Nocardia asteroides* in cows. Mn. Vet. Med. **24**: 89 – 97.

Williams, C. R. (1995). Diseases of Dairy Cattle 1st ed. Philadelphia: A lea and Febiger Book. Williams and wilkins. Pp. 287.

Williams, S. T. Goodfellow, M. ; Aderson, G. ; Wellington, E. M. H. ; Sneath, P. H. A. and Sackin, M. J. (1983). Numeric1 al classification of streptomyces and related genera. J. Gen. Microbio.**29**: 1743 – 1813.

Yassin, E. T. and Hussein, M. A. (1995). A preliminary survey of the causal organisms of mastitis in goats. S. J. Vet. Sci. and Anim. husb. **25**: 75-80.

APPENDICIES

Appendix A. Media

(a) Tryptic Soy Agar (Difco)

Tryptic soy agar 40g, distilled water 1 litter; pH 7.2. Autoclaved at 121℃ for 15 minutes.

(b) Nutrient agar (Oxoid)

Nutrient agar 28g; distilled water 1 litre; pH 6.8; autoclaved at 121° C for 15 minutes.

(c) Xanthine medium agar

Xanthine, 4g; Nutrient agar, 1 litre; autoclaved at 121° C for 15minutes.

(d) Tyrosine medium agar

L–tyrosine, 4g; Nutrient agar, 1 litre; autoclaved at 121°C for 15 minutes.

(e) Starch medium agar

Starch 10g; Nutrient agar, 1 litre ; autoclaved 121° C for 15 miutes.

(f) Casein medium

100 ml of skimmed milk powder (Oxoid) (10%, W/V) was autoclaved at 121° C for 15 minutes; add to sterile molten T.S. A, to give final a concentration of 1%, W/ V.

(g) Urea medium

2.4g urea agar base; 95ml distill water. Sterilized by autoclaving at 121°C for 5minutes. Then 2g of urea crystal was added to 5 ml distilled water. The preparation was dispersed in bottle and allowed to set in slop position.

(h) Peptone Water Sugars

900 ml peptone water was adjusted to pH 7.1 – 7.3 so that the addition of 10 ml Andrade's indicator would bring it to pH 7.5. The preparation was then sterilized at 121° C 20 minutes. 5 – 10g of the appropriate Sugar was dissolved in 90ml of peptone water, steamed for 30 minutes, distributed into 5 ml volumes in test tubes and sterilized at 110° C for 10 min.

Appendix B. Stains

a) Gram's stains

A culture smear was spread on a microscopic slide, fixed by gentle flaming and put on a glass holder. The slides then flooded with crystal violet stain for one minute, washed with tap water. The slides coerd with iodine and washed with tap water The slides were decolorized with acetone for 15 seconds and slides were washed with tap water. The slides were stained with diluted carbol fuchsin for one minute and washed with water again and allowed to dry.

A drop of immersion oil was added to each slide and examined under microscope.

B) Modified Ziehl - Neelsen's stain

After fixation of the slide, it was flood with dilute carbolfuchsin for 15 minutes, and then the slides were washed thoroughly under running water. Decolorized in acetic acid 5% for 15 seconds, it was washed well in water. When decolorized was complete, counter stain with methylene blue, washed and dry.

C) Newman's stain

This was prepared accounting to Carter (1979).

Staining solution:

Methylene blue	2gm
Ethyle alcohol (95%)	60ml
Xylene C.P	40ml

Acetic acid glacil 6ml

Methylene blue was dissolved in warm alcohol xylene was added then the mixture was filtered and kept in a bottle.

Staining Procedure:

- Immerse smear for about one minute.
- Remove and drain until dry.
- Wash thoroughly with water.

Appendix C. Disinfection and sterilization

A) Disinfection

Ethyl alcohol 96% was used to disinfect the surfaces of laboratory desks before and after each laboratory work. 70% alcohol was used as disinfectant for teats udder

B) Sterilization

- Petri dishes, graduated pipettes and test tubes were sterilized in a hot air oven at 160℃ for one hour.
- Flasks, test tube, bottles and other glass containers were sterilized in the autoclave for 15 minutes at 121℃ and 15lb.
- Culture media sterilized by autoclaving at 121℃ for 15 minutes.
- Carbohydrate media was sterilized by autoclaving for 15 minutes at 110℃.

Appendix D. Somatic cell count (SCC)

The direct microscopic somatic cell count was applied as recommended by Packard et al. (1992).

- Milk sample (0.01ml) was prepared over an area of a glass slide. The milk samples were warmed immediately before transferring them to slides.
- The smears were dried and heated.
- The smears were staining with Newman's stain (according to Carter, 1979).
- The total number of cell count was calculated using the following formula:

$$\text{Number of leukocyte per ml of milk} = \frac{\text{Number of leukocytes counted}}{\text{Number of field counted}} \times \text{MF}$$

- The magnification factor calculated was found to be :

$$\frac{40000}{3.1416 \times (1.7 \times 1.7)} = 440.556$$

Appendix E. Local treatment

1) Neomastipra -JR5 (Laboratory Hipra, S. A, Spain)

Composition per syringe of this drug is as follow:

1. Benzyl penicillin procaine 100.000 I. U.
2. Dihydrostreptomycin (sulphate) 62.4mg.
3. Neomycin (sulphate) 36mg.
4. Polymycin B (sulphate) 50000I. U.
5. Sulphadimidine (sodium) 250mg.
6. Sulphathiazole 250mg.
7. Hydrocortison.

a)Administration rout

Intra mammary infusion.

B) Dosage

Half of the syringe per affected quarter every twelve hours for three consecutive days (six administrations).

c) Withdrawal period

Milk three days (six milking).

d) Special precautions

1. The affected half was treated after careful milking, cleaning and disinfection of the teat, the equipment and milk man's hands.
2. Empting the mammary gland was avoided after infusion in order not to lose the infused drugs, so milking of the treated goat was not done with in six

hours after treatment. Treatment was taken immediately after milking and preferably in the evening.

3. Only affected half was treated.

4. How to apply the intra mammary infusion:

The cap was removed from the end of the nozzle of the injector and the nozzle was inserted carefully into the opening of the teat. Then the content of the injector was squeezed slowly in the teat with careful massage of the teat to disperse the suspension upward into the quarter tissues and cavities.

2) Gentamast (Bremer pharma GMBH 27540 Bremer haven, Germany).

a) Composition

1 syringe (intra mammary injector) with 7.5g contains gentamycin sulphate 170mg (equal to gentamicin 100mg).

b) Dosage

Half of the syringe per infected quarter. Clean and disinfectteats and milk out the udder prior to treatment.

Repeat the instillation after 24hours up 3 consecutive days (three administrations).

d) Withdrawal period

Milk two days (4 milking).